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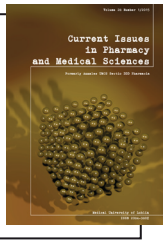

The influence of montelukast on the autonomic nervous system activity in rats with cyclophosphamide-induced hemorrhagic cystitis

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ABSTRACT

The complex pathogenesis of cyclophosphamide-induced hemorrhagic cystitis involves arachidonic acid-derived inflammatory mediators, among them leukotrienes. Montelukast, a leukotriene receptor antagonist, is reported to exert an alleviatory effect in the course of cystitis associated with overactive bladder symptoms. The aim of this study was to verify whether the effect of montelukast is also associated with its influence on autonomic activity. The experiment included 20 rats with cyclophosphamide-induced hemorrhagic cystitis (75 mg/kg, four doses every second day), among them, 10 treated with oral montelukast (10 mg/kg for 8 days) and 10 controls. Time and frequency domain analyses of heart rate variability (HRV) were conducted in all the rats as an indirect measure of their autonomic activity. The montelukast-treated animals showed an increase in root mean square of successive differences (rMSSD), as well as an increase in HRV spectrum total power (TP) and power of very low (VLF) spectral component. This suggests that due to its anti-inflammatory and its anti-leukotriene effect, montelukast improves overall autonomic activity, with no preferential influence on either the sympathetic or parasympathetic part. Furthermore, the increase in VLF corresponds to attenuation of inflammatory response. In conclusion, this study showed that aside from its antagonistic effect on leukotriene receptors, montelukast can also modulate autonomic activity.

INTRODUCTION

Hemorrhagic cystitis is a clinical condition characterized by irritating voiding symptoms, urinary frequency, dysuria, urgency, strangury, suprapubic discomfort, evident pain, microhematuria or even potentially life-threatening hematuria. Hemorrhagic cystitis may result from bacterial and viral infections, or develop as a side effect of radio- and chemotherapy [18]. Cyclophosphamide – CP (2-[bis(2-chloroethyl) amino]tetrahydro-2H-1,2,3-oxazaphosphorine 2 oxide) is an essential antineoplastic agent which may promote the development of hemorrhagic cystitis. This cytostatic agent is still used in the treatment of B cell-derived malignancies and some solid tumors, as well as in conditioning before bone marrow transplantation and in the pharmacotherapy of some immune-inflammatory diseases, such as rheumatoid arthritis and systemic lupus erythematosus. Cyclophosphamide-induced hemorrhagic cystitis (CP-HC), an important adverse reaction to cyclophosphamide, occurs in up to 75% of patients receiving high doses of this agent [18]. Despite the high incidence of CP-HC, its pathophysiology is still unclear. The cascade of events involved in the pathogenesis of CP-HC is generally consistent with that observed in the case of interstitial cystitis (painful bladder syndrome) [12]. However, in contrast to interstitial cystitis, an idiopathic disease with an unknown trigger for inflammatory response, development of CP-HC is stimulated by toxic 4-hydroxy metabolites of cyclophosphamide formed in the liver (and perhaps also in the kidneys), especially acrolein. This highly reactive and harmful aldehyde accumulates in the bladder and may induce urothelial damage due to a direct interaction with umbrella cells [18]. Detailed description of consecutive stages of CP-HC pathogenesis, as well as the data on the role of immune-inflammatory cells and complex paracrine and neuroendocrine background of the process, were presented elsewhere [1], also in our paper [9]. Arachidonic acid derivatives, prostanoids and leukotrienes, are one of the most important inflammatory mediators. In recent work, the

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role of prostanoids in urinary bladder function under normal and pathological conditions is increasingly discussed. Both 1 and 2 cyclooxygenase isoforms are found in the bladder, and prostaglandins are generally postulated to contribute to bladder overactivity. Consequently, prostaglandin-targeting agents are increasingly postulated as a novel pharmacological approach to bladder dysfunction [24]. In contrast, little is known about the role of leukotrienes in CP-HC pathogenesis. Leukotrienes (LT), released to the bladder wall from mast cells, along with other granule-stored preformed mediators (e.g. heparin, histamine), are newly synthesized potent lipid inflammatory mediators. LT were shown to be among the most potent chemotactic agents, and are factors contributing to increased permeability and vasoactive reactions. Several chronic inflammatory conditions, such as dermatitis, arthritis and unspecific inflammatory bowel diseases (all predominantly associated with LT class B4), asthma, allergic rhinitis and nephritis (mainly associated with LT class C4) have been linked to LT overproduction [13]. Bouchelouche *et al*. demonstrated an increase in the urinary excretion of LT class E4 in patients with interstitial cystitis [3]. The same authors found leukotriene receptors in bladder tissues [4], and hypothesized that anti-leukotriene agents may constitute a novel treatment for cystitis. This hypothesis was confirmed empirically [5], as 10 women diagnosed with interstitial cystitis showed a significant decrease in 24-hour urinary frequency, nocturia and pain after a 1-month treatment with the leukotriene receptor antagonist, montelukast; these beneficial effects persisted for the 3 months of the treatment. The efficacy of montelukast (MLKT) in the control of bladder overactivity was also reported by Traut *et al*. [27], who demonstrated that administration of this agent to a patient with interstitial cystitis and concomitant allergic rhinitis resulted in substantial attenuation of urinary urgency and pain; however, the effect was no longer observed after discontinuation of MLKT treatment. Altogether, these data point to anti-leukotriene treatment as an interesting pharmacological approach to cystitis.

Taking into account the premises mentioned above, we decided to clarify whether the beneficial anti-leukotriene effect of montelukast observed in patients with bladder overactivity is also associated with its influence on the autonomic nervous system (ANS) activity. Therefore, the aim of this study was to analyze autonomic activity, determined on the basis of heart rate variability (HRV), in montelukast-treated rats with cyclophosphamide-induced hemorrhagic cystitis.

MATERIALS AND METHODS

All the hereby presented experiments were performed in line with the National Institute of Health Guidelines for the care and use of laboratory animals, and the European Council Directive of 22 September 2010 for Care and Use of Laboratory Animals (2010/63/UE). The protocol of the study was approved by the First Local Ethics Committee in Krakow.

The experiment included 20 eight-week-old albino Wistar rats with mean initial body weight of 198.4 ± 7.9 g, obtained from the central animal house at the Faculty of Pharmacy UJCM. Upon admission to the local animal house at the Department of Pathophysiology, the animals acclimated to new living conditions for one week. During this period, the rats were housed under standard conditions, five individuals per cage, with unlimited access to water and food (Labofeed, Kcynia). The first stage of the proper experiment comprised the measurement of daily water and food intake, determined with an aid of individual metabolic cages. The following day, the rats were randomized to either the control group (group 1, n=10) or to the group treated with the oral leukotriene receptor antagonist, montelukast (group 2, n = 10). On the 1st, 3rd, 5th and 7th day of the experiment, body weight of the rats was determined and then each animal received an intraperitoneal injection of cyclophosphamide (75 mg/kg). This dosage regimen, subject to minor modifications, has been used to induce chronic CP-HC in many previous studies [6,10]. Moreover, animals from group 2 received MLKT dissolved in an individually predetermined daily volume of water. A commercially available preparation containing MLKT was used (Montelukast Sandoz, 500-mg sachets containing 4 mg of active substance). MLKT was administered at 2 mg per rat per day. Effective doses of MLKT were calculated, adjusted for potential changes in body weight, and, additionally, corrected for daily intake of water the case of montelukast (after 1st CP dose – 9.91 mg/kg, after 2nd CP dose – 10.32 mg/kg, after 3rd CP dose – 10.66 mg/kg and after 4th CP dose – 11.05 mg/kg). MLKT was continued for 7 days, i.e. till the end of CP treatment. The mean cumulative dose of MLKT administered throughout the 7-day treatment period was 10.49 mg/kg, which was consistent with the cumulative dose of MLKT used by other authors (10 mg/kg orally) [19].

Both control and MLKT-treated rats were kept in individual cages, with limited access to water and unlimited access to food. All animals survived till the end of the treatment, but their condition deteriorated gradually with subsequent CP doses. On the 7th day of the experiment, after receiving the last dose of CP, all animals were placed back in single metabolic cages with still maintained, predetermined access to water (with dissolved MLKT in the case of group 2) to estimate their food intake, final body weight and 24-hour urine output.

On the 8th day, 20-minute resting ECG recordings were obtained from all animals under general pentobarbital anesthesia (Morbital, 60 mg pentobarbital sodium per kg body weight), and subjected to time and frequency domain analyses of heart rate variability (HRV).

The ECG signal used for HRV analysis was collected utilizing Ag/AgCl pediatric electrodes (EK-S30 Sorimex PSG) and Polygram System (ADInstruments) hardware and software, following hair removal, application of abrasion paste and standard ECG gel.

HRV is an analysis of normal-normal heart beat intervals in sinus rhythm, as they are known to be modulated by the ANS [15,22,25]. The following parameters were calculated on the basis of the obtained ECG recordings:

1) standard time parameters: mean, max and min NN intervals, standard deviation of all NN intervals (SDNN), root mean square of successive differences (rMSSD) (all in ms), mean heart rate (HR, 1/min), and

2) spectral (frequency) parameters: total HRV spectrum power (TP), power of very low frequency component (VLF), power of low frequency component (LF) and power of high frequency component (HF) (all in ms2). Moreover, we normalized the HRV spectrum for VLF so as to obtain two normalized parameters, nLF and nHF, both expressed in normalized units (n.u.) [15,22,25].

Similar to previous studies (e.g. Goncalves *et al*. [11]: 0.10 < LF < 1.0 <HF < 3.0), the spectrum bands for respective components were set at 0.18 < VLF < 0.28 < LF < 0.78 < HF <3.

Once HRV recordings had been taken, a lethal dose of sodium pentobarbital was administered to each animal (160 mg pentobarbital sodium per rat), and urinary bladders were collected to determine their absolute wet weight (BWW) and its proportion in final body weight. The bladders were removed along with the proximal urethra, carefully emptied and immediately thereafter weighted on an analytical scale. According to literature, the severity of cystitis may be evaluated not only on the basis of macroscopic and microscopic changes in the urinary bladder, but also on the basis of BWW. Therefore, BWW is considered to be an indirect marker of cystitis and bladder dysfunction [28]. After determining BWW, the bladders were fixed in 4% formalin + PBS solution and sent for histopathological examination. Routinely prepared microscopic slides stained with hematoxylin and eosin were examined for the severity of inflammatory lesions.

The significance of intergroup differences related to body weight, bladder wet weight, average daily water intake and daily urine excretion was verified with parametric Student t-test – with the threshold of significance set at p=0.05.

The calculated HRV results were submitted to two-stage statistical analysis. In the first step, the non-parametric Mann-Whitney test was performed with the calculation of both tested statistics (U and Z values). As none of the HRV parameters were distributed normally, they were subjected to logarithmic transformation prior to the next step of the statistical analysis based on the t-Student test. We adopted final statistical significance if both tests demonstrated p <0.05 for the estimated parameter.

RESULTS

Both groups of rats showed progressive weight loss after administration of successive CP doses. Indeed, significant intergroup difference in body weight was observed solely after administration of the 1st CP dose. Considering intragroup body weight changes, we found that the starting and final differences were statistically significant (p<0.05) in both groups.

However, the two groups did not differ significantly in terms of their BWW and final 24-hour urine outputs, although slightly lower values of the latter parameter were observed in the case of MLKT-treated rats.

Detailed characteristics of rats from the two groups are given in Table 1. All results are presented as mean values ± SD.

Table 1. The characteristics of the studied animals – CP-HC model. Statistical analysis was performed for intergroup differences

	Group 1 control CP-HC rats	Group 2 CP-HC rats + MLKT	Statistic Group 1-2
starting body weight [g]	198.4±7.9		
body weight after CP 1st dose [g]	188.0±11.3	201.9±12.3	p=0.03
body weight after CP 2nd dose [g]	184.5±9.1	193.9±11.3	NS
body weight after CP 3rd dose [g]	183.7±10.5	188.8±10.0	NS
body weight after CP 4th dose [g]	180.0±11.4*	181.0±14.1*	NS
average daily water intake [ml]	22.0±2.3	25.1±4.1	NS
bladder wet weight [mg]	131.7±25.6	121.5±24.3	NS
bladder wet weight related to final body weight [%]	0.073±0.01	0.067±0.01	NS
24-hour urine excretion day after 4th CP dose [ml]	14.8±6.4	10.3±5.9	NS

Footnotes: CP-HC – cyclophosphamide-induced hemorrhagic cystitis, **CP** – cyclophosphamide, **MLKT** – montelukast, **NS** – non significant, ***** – intragroup (1-1; 2-2) statistically significant difference (p < 0.05) – considering the starting and final values

HRV analysis

The two groups did not differ significantly in terms of their parameters determined on time domain HRV analysis, although rMSSD reached a statistically insignificant higher value in MLKT-treated rats than in the controls.

Spectral domain HRV analysis revealed intergroup differences in the HRV spectrum, yet only the TP and VLF results met the assumption of statistical significance. In other cases, we have shown a partial statistical significance (in the Mann-Whitney test), but which was unconfirmed in the t-Student test. Both total power (TP) and power of very low (VLF) spectral component were higher in the MLKT-treated rats. The values of remaining non-normalized spectral domain parameters did not differ significantly between the groups, although both LF and HF were slightly higher in MLKT-treated animals. What is more, the values of both normalized parameters, nLF and nHF, did not differ significantly between the groups, despite an insignificant tendency to higher nLF and lower nHF in MLKT-treated rats.

The statistical estimation is presented in Table 2 and the detailed results of time and spectral domain HRV analyses are revealed in Table 3. All results are presented as mean values ± SD.

Table 2. The results of the statistical estimation

HRV parameter	Mann-Whitney test		t-Student test
	U statistic	Z statistic	
Time-domain parameters			
mean NN	p<0.05	NS	NS
max NN	NS	NS	NS
min NN	NS	NS	NS
range	p<0.05	NS	NS
average HR	p<0.05	p<0.05	NS
SDNN	NS	NS	NS
rMSSD	p<0.05	NS	NS
Spectral-domain parameters			
TP	p<0.05	p<0.05	p<0.05
VLF	p<0.05	p<0.05	p<0.05
LF	NS	NS	NS
HF	NS	NS	NS
nLF	p<0.05	p<0.05	NS
nHF	p<0.05	p<0.05	NS

Table 3. Time- and spectral-domain HRV analysis parameters obtained from 20-minute ECG recordings in rats – CP-HC model

	CP-HC rats control	CP-HC rats treated with MLKT	Final statistic estimation
TIME-DOMAIN HRV ANALYSIS			
mean NN [ms]	158.81±11.66	167.64±13.27	NS
max NN [ms]	189.91±2.52	188.79±3.52	NS
min NN [ms]	144.19±6.44	143.02±5.59	NS
range [ms]	42.74±8.25	45.74±5.43	NS
average HR [bpm]	380.15±26.93	359.87±26.68	NS
SDNN [ms]	7.44±1.99	10.07±4.29	NS
rMSSD [ms]	6.27±4.07	15.82±11.16	NS
SPECTRAL-DOMAIN HRV ANALYSIS			
TP [ms2]	17.39±15.55	45.23±37.10	p=0.05
VLF [ms2]	11.26±9.57	36.20±31.18	p=0.05
LF [ms2]	2.79±2.31	4.35±3.59	NS
HF [ms2]	4.49±3.97	6.14±5.37	NS
nLF [n.u.]	39.54±14.23	50.06±15.51	NS
nHF [n.u.]	60.40±14.23	49.94±15.51	NS

Footnotes: CP-HC – cyclophosphamide-induced hemorrhagic cystitis, **MLKT** – montelukast, **HRV** – heart rate variability, **mean NN** – mean normal-normal interval [ms], **max NN** – maximal normal-normal interval [ms], **min NN** – minimal normal-normal interval [ms], **range** – mean difference between max and min intervals [ms], **average HR** – average heart rate [bpm – 1/min], **SDNN** – total standard deviation of all normal-normal intervals [ms], **rMSSD** – root mean square of successive differences, **TP** – total power of HRV spectrum, **VLF** – power of very low frequency component in the HRV spectrum, **LF** – power of low frequency component in the HRV spectrum, **HF** – power of high frequency component in the HRV spectrum, **nLF** – normalized LF, **nHF** – normalized HF, **NS** – non-significant

Histopathological analysis

The specimens from the bladders of control rats with experimentally induced CP-HC showed the presence of chronic, partially purulent, inflammatory infiltrate. Moreover, the bladder wall was edematous and hyperemic, with small amount of non-specific granulomatous tissue.

Only two specimens from the bladders of MLKT-treated rats showed a mild degree edema, and a trace of chronic inflammatory infiltrate was found in only one specimen. The remaining bladder specimens from MLKT-treated animals did not show any abnormalities upon histopathological evaluation.

DISCUSSION

The development of the CP-HC model is related to the adverse, systemic CP action, including body weight loss. The reason of the progressive body weight reduction was repetitive CP dosage regimen and cumulative, cytotoxic CP effect due to its action affecting not only proliferating cancerous cells, but also healthy ones undergoing rapid division (e.g. gastrointestinal epithelial cells). The CP -evoked mucositis of the alimentary tract with possible ulceration leads to impaired nutrient absorption and finally results in malnutrition [21]. Moreover, CP was also reported to cause taste alternation (reduced taste sensitivity – hypogeusia, or even complete loss of taste – ageusia) and it can also lead to reduced food intake [20]. Indeed, considering all of the study animals together, we showed a diminished daily food consumption at the end of the experiment (20.8 ± 3.9 g), compared to the initial value assessed in the beginning (59.3 ± 8.3 g; $p<0.05$). Furthermore, we found some significant changes in the HRV spectra of rats with cyclophosphamide-induced hemorrhagic cystitis that had been treated with montelukast (a leukotriene receptor antagonist). This was, namely, an increase in total spectrum power (TP) and power of very low (VLF) spectral component. Moreover, histopathological abnormalities found in the specimens from MLKT-treated rats were markedly less evident than in the controls.

TP is generally considered a marker of global autonomic tone and excitability. The physiological meaning of the VLF effect, however, still remains unclear, although this parameter accounts for about 90% of the HRV spectrum [22]. The origin of VLF is complex; this parameter is postulated to be a marker of various circadian and neuroendocrine rhythms, thermoregulatory processes, renin-angiotensin system activity and hemodynamic feedback delays. Although VLF cannot be unambiguously linked to any of the two ANS arms, as a major marker of physical activity and general stress, it is considered to be a measure of sympathetic, rather than parasympathetic tone [15,25]. According to some authors, VLF reflects severity of inflammation, and it negatively correlates with the level of pro-inflammatory cytokines [14,23]. In addition, some researchers have found significant inverse correlations between inflammatory biomarkers, e.g. C-reactive protein (CRP), and selected HRV metrics derived from both time and frequency domains, especially SDNN and VLF [16,17].

In our previous studies, we have assessed the autonomic nervous system activity in rats with a "pure" model of CP-HC, also using HRV methodology. Therein, we revealed that CP-HC rats were characterized by a statistically significant decrease of HRV total power and power of all the non-normalized, basic components: VLF, LF and HF, compared to intact animals. In such work, we concluded that cyclophosphamide reduced global autonomic activity and the diminished VLF power, together, might be considered as an indirect HRV marker of systemic inflammation evoked by CP [7,8].

The spectral HRV analysis of the CP-HC animals treated with montelukast, performed in the present experiment, showed opposite results – a significant increase of VLF and TP was demonstrated. In our opinion, therefore, the applied MLKT dose was sufficient to exert a modulatory effect on the autonomic activity (which was confirmed by the VLF and TP changes in HRV spectrum), but not sufficient to alleviate the adverse CP systemic effects which contributed to body weight loss and general deterioration of the study animals.

In line with widely accepted international HRV interpretation guidelines [22], the increase in VLF of MLKT-treated rats might result from the anti-inflammatory effect of this agent, specifically, the blockage of leukotriene receptors and the functional inhibition of these pro-inflammatory lipid mediators. This, in turn, led to improvement of global autonomic activity, as shown by increased TP.

There is a link between inflammatory mediators and autonomic activity. Chemical mediators are equally effectors of inflammation and signal molecules for both autonomic and somatic afferent fibers. The neural immunomodulation pathway is potentiated through the direct, humoral action of

circulating proinflammatory cytokines, reaching the central neuronal structures. [2]. Conversely, within the autonomic nervous system, through the release from efferent terminals, the main neurotransmitters, together with a variety of co-transmitters, acting via receptors present on the surface of immune cells, can also affect the function of the immunocompetent cells. Thus, there is a strong, neuroimmunological interdependence [2].

Montelukast has been reported to exert additional, anti-inflammatory properties. These include 5-lipooxygenase inhibition, antagonizing the effects of agents acting via purinergic P2Y receptors, inhibition of pro-inflammatory cells adhesion and migration [26]. MLKT has also an ability to diminish pro-inflammatory mediators and that action indirectly contributes to the modulation of the autonomic activity. This effect results from MLKT-evoked cAMP elevation due to the adenosine 3,5-cyclic monophosphate phosphodiesterase (PDE) inhibition [26]. Elevated cytosolic Ca level is a necessary condition for neutrophil stored mediators degranulation and for the production of lipid mediators. Cyclic AMP promotes a restoration of calcium cellular homeostasis in neutrophils and other cells, thus cAMP-elevating agents such as montelukast may provide an additional, indirect anti-inflammatory effect due to the decrease of some cellular mediators release [26].

Our findings suggest that montelukast, apart from the aforementioned VLF effect, may also change some remaining HRV parameters. However, owing to the ambiguous profile of changes in rMSSD, LF, HF, nLF and nHF of MLKT-treated rats, we were unable to conclude if this agent induces any shift in the sympathetic/parasympathetic balance. According to HRV interpretation standards [22], rMSSD and HF are pure markers of parasympathetic activity. In turn, LF is influenced by both sympathetic and parasympathetic tone, although the effect of the former is somehow stronger. Therefore, HRV spectra are usually normalized to obtain nLF and nHF values, being, respectively, pure markers of sympathetic and parasympathetic activity [15,22,25]. Although our MLKT-treated rats showed a trend of increasing rMSSD, this was not associated with concomitant changes in non-normalized and normalized spectral parameters, HF and nHF. However, we also did not find a significant intergroup difference in sympathetic activity measure, nLF. Altogether, these findings suggest that MLKT does not cause a shift in the sympathetic/parasympathetic balance. Yet, the fact that MLKT-treated rats showed a tendency to higher LF and HF, and demonstrated slightly lower nLF and nHF values than the controls, justifies further research on the influence of montelukast on the sympathetic/parasympathetic balance. At present, we can only state that this agent enhances global autonomic activity, and an increase in the VLF power of the HRV spectrum may reflect lower severity of inflammation.

CONCLUSIONS

MLKT treatment resulted in an improvement of the histological abnormalities revealed in the bladder assessment of the CP-HC animals.

Montelukast seems to exert an anti-inflammatory effect during the course of CP-HC. This was not only due to leukotriene receptor antagonism, but also due to indirect stimulation of autonomic activity.

An increase in VLF power of the HRV spectrum observed after administration of montelukast may be considered additional, indirect evidence for the anti-leukotriene and anti-inflammatory effect of this agent.

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REFERENCES

1. Altayli E. et al.: Prevention and treatment of cyclophosphamide and ifosfamide-induced hemorrhagic cystitis. *J. Mol. Pathophysiol.,* 1, 53, 2012.
2. Benarroch E.E.: Autonomic-mediated immunomodulation and potential clinical relevance. *Neurology,* 73, 236, 2009.
3. Bouchelouche K. et al.: Increased urinary leukotriene E4 and eosinophil protein X excretion in patients with interstitial cystitis. *J. Urol.,* 166, 2121, 2001.
4. Bouchelouche K. et al.: The action of cysteinyl-leukotrienes on intracelluar calcium mobilization in human detrusor myocytes. *BJU Int.,* 87, 690, 2001.
5. Bouchelouche K. et al.: The cysteinyl leukotriene D4 receptor antagonist montelukast fort the treatment of interstitial cystitis. *J. Urol.,* 166, 1734, 2001.
6. Chuang Y.C. et al.: Intravesical botulinum toxin A administration inhibits COX-2 and EP4 expression and supresses bladder hyperactivity in cyclophosphamide-induced cystitis in rats. *Eur. Urol.,* 56, 159, 2009.
7. Dobrek Ł., Baranowska A., Thor P.J.: The influence of oxazaphosphorines alkylating agents on autonomic nervous system activity in rat experimental cystitis model. *Acta Pol. Pharm.,* 70, 1097, 2013.
8. Dobrek Ł., Thor P.: Heart rate variability in overactive bladder experimental model. *Arch. Med. Sci.,* 9, 930, 2013.
9. Dobrek Ł., Thor P.J.: Bladder urotoxicity pathophysiology induced by the oxazaphosphorine alkylating agents and ist chemoprevention. *Postepy Hig. Med. Dosw. (online),* 66, 592, 2012.
10. Girard B.M. et al.: Increased expression of interleukin-6 family members and receptors in urinary bladder with cyclophosphamide-induced bladder inflammation in female rats. *Front. Neurosci.,* 5, article 20, 2011.
11. Goncalves H. et al.: Analysis of heart rate variability in a rat model of induced pulmonary hypertension. *Med. Eng. Phys.,* 32, 746, 2010.
12. Grover S. et al.: Role of inflammation in bladder function and interstitial cystitis. *Ther. Adv. Urol.,* 3, 19, 2011.
13. Haeggstrom J.Z., Funk C.D.: Lipooxygenase and leukotriene pathways: biochemistry, biology and roles in disease. *Chem. Rev.,* 111, 5866, 2011.
14. Huston J.M., Tracey K.J.: The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy. *J. Intern. Med.,* 269, 45, 2011.
15. Karim N, Hasan JA, Ali SS. Heart rate variability – a review. *J. Basic Appl. Sci.,* 7, 71, 2011.
16. Lampert R. et al. : Decreased heart rate variability is associated with higher levels of inflammation in middle-aged men. *Am. Heart J.,* 156, 759e1, 2008.

17. Lanza,G.A. et al.: Relation of heart rate variability to serum levels of C reactive protein in patients with unstable angina pectoris. *Am. J. Cardiol*., 97,1702, 2006.
18. Lee G., Romih R., Zupancic D.: Cystitis: from urothelial cell biology to clinical application. *Biomed. Res. Int*., article ID 473536, 2014.
19. Mohamadin A.M. et al.: Montelukast, a leukotriene receptor antagonist abrogates lipopolysaccharide-induced toxicity and oxidative stress in rat liver. *Pathophysiology,* 18, 235, 2011.
20. Mukherjee N. et al.: Pre-treatment with amifostine protects against cyclophosphamide-induced disruption of taste in mice. *PLoS ONE,* 8, e61607, 2013.
21. Nicolini A. et al.: Malnutrition, anorexia and cachexia in cancer patients: a mini review on pathogenesis and treatment. *Biomed. Pharmacother.,* 67, 807, 2013.
22. No Authors listed. Heart rate variability. Standards of measurement, physiological interpretation and clinical use. Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology. *Eur. Heart J.,* 17, 354, 1996.
23. Papaioannou V., Pneumatikos I., Maglaveras N.: Association of heart rate variability and inflammatory response in patients with cardiovascular diseases: current strengths and limitations. *Front. Physiol.,* 4, 174, 2013.
24. Rahnama'i M.S., van Koevringe G.A., van Kerrebroeck P.E.: Overactive bladder symptom and the potential role of prostaglandins and phosphodiesterases: an introduction. *Nephro. Urol. Mon.,* 5, 933, 2013.
25. Sztajzel J.: Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system. *Swiss. Med. Wkly.* 134, 514, 2004.
26. Tintinger G.R. et al.: Montelukast: more than a cysteinyl leukotriene receptor antagonist? *ScientificWorldJournal,* 10, 2403, 2010.
27. Traut J.L. et al.: Montelukast for symptom control of interstitial cystitis. *Ann. Pharmacother.,* 45, e49, 2011.
28. Zeng J. et al.: Cause of residual urine in bladder outlet obstruction: an experimental study in the rat. *J. Urol.,* 188, 1027, 2012.